Title- Investigation of Therapeutic Potential of Telomerase Inhibitors in Ovarian Cancer

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Summary

Telomerase is a very attractive target for therapy, due to its specific presence in cancer cells and undetectable expression in somatic cells. Telomerase inhibitors have been investigated as potential cancer therapy for their acute cytotoxic effects as they halt the proliferation of cancer cells by telomere uncapping and activating the DNA damage response pathway. p53 is an essential protein with tumor suppressor functions that has a pivotal role in DNA damage. It is frequently found to be mutated in various types of cancers. Deregulation of p53 plays a role in determining the sensitivity of cancer toward anti-cancer compounds which cause DNA damage. However, the relationship between telomerase inhibitors and the tumor suppressor protein p53 is not completely understood. In this study, we investigated the cytotoxic effects of MST-312, BIBR1532, and 6-thio-dG in ovarian cancer cells (OCCs) with different p53 statuses and studied the combinatorial effect of a telomerase inhibitor, MST-312, and a plant-based flavonoid, quercetin in cancer cells.

Our results indicate that short-term acute treatment with MST-312, BIBR1532, and 6-thio-dG exhibits p53-dependent cytotoxicity in a panel of ovarian cancer with variable p53 expression. However, in isogenic p53+/+ and p53-/- ovarian cancer cell lines created using CRISPR/Cas9, MST-312 exhibits p53-dependent cytotoxicity while BIBR1532 and 6-thio-dG exhibit p53 independent cytotoxicity. Moreover, MST-312 treatment effectively aggravated colony-forming ability in a p53-dependent manner. Additionally, the re-introduction of p53 in p53-/- cells restored their sensitivity towards MST-312. Since p53 essentially determines the cell cycle progression and induces cell cycle arrest and/or apoptosis in response to genomic damage, we investigated the effect of MST-312, BIBR1532, and 6-thio-dG on cell cycle progression. MST-312 induced S phase cell cycle arrest followed by cell death in the presence of p53 while in the absence of p53, it led to a more significant G2/M phase arrest. BIBR1532 induced S/G2/M phase arrest in p53+/+ cells but similar cell death irrespective of the p53 status of the cells. 6-thio-dG induced S phase cell cycle arrest in the presence of p53 while in the absence of p53, it led to a more significant G2/M phase arrest but similar cell death irrespective of the p53 status of the cells. Further, we investigated the mechanism of cell death of MST-312 and BIBR1532 in ovarian cancer cells and found that MST-312 induced p53-dependent

apoptosis while BIB1532 induced p53-independent necrosis. Gene expression analysis revealed that MST-312 modulates the expression of p53 target genes, such as anti-apoptotic genes (Fas and Puma) and cell-cycle markers (p21, cyclin B, cyclin Ds) confirming the observed cell cycle and apoptosis phenotypes. Additionally, long-term treatment with MST-312 and BIBR1532 results in p53-independent telomere shortening in telomerase-positive cancer cells. Interestingly, long-term treatment with 6-thio-dG led to an increase in telomere length in p53-positive cells.

Collectively our results provide evidence of distinct anti-cancer effects of telomerase inhibitors and shed light on their molecular mechanisms and treatment outcomes, particularly in terms of p53 expression status in cancer cell lines.

Furthermore, when combined with other anticancer agents, telomerase inhibitors can improve efficacy and provide synergistic effects, offering a promising strategy for fighting cancer. In this study, we investigated the synergistic response of quercetin and MST-312 as a combinatorial therapy, to reduce the required therapeutic dosage of individual drugs in cancer cells. We observed that at low doses, MST-312 and quercetin exhibit a significant synergistic anti-cancer effect. Co-treatment of quercetin and MST-312 significantly reduced colony formation ability and increased the proportion of apoptotic cells. We further studied the effect of the combination on DNA damage response proteins. Co-treatment with quercetin and MST-312 led to a significant increase in the expression of p53, p21, and γ-H2AX. Furthermore, we found that combining MST-312 and quercetin led to a 1000-fold inhibition of telomerase activity. The gene expression of the homology repair pathway, ATM, and RAD50 was also measured and found to be significantly lower in the combination group. Our findings show that MST-312 in combination with quercetin increases DNA damage leading to apoptosis. This is accomplished by inhibition of telomerase activity and suppression of DNA repair mechanisms. These findings hold great potential for enhancing the efficacy of cancer therapeutics since the combination of MST-312 and quercetin specifically targets cancer cells at low therapeutic doses of each compound.